Amendments to the Specification

In Example 2, delete the two paragraphs at page 23 line 5 to page 25, line 36, and substitute the following paragraphs:

The maize expression vector, pUGN81-3, containing the ubiquitin promoter regulatory element driving the β-glucuronidase gene was used as disclosed herein. Plasmid pUGN81-3 was a 8730 base pairs double stranded plant transformation vector composed of the following sequences in clockwise order. Nucleotides 1 to 17 encoded a polylinker ~~having the sequence AGCTTCCGCG GCTGCAG~~. Nucleotides 18 to 2003 of pUGN81-3 were the maize ubiquitin promoter and first intron thereof and were PCR amplified from genomic DNA of maize genotype B73 (Christensen et al., (1992) Plant Mol. Biol. 18:675-689). Nucleotides 2004 to 2022 of pUGN81-3 encoded a polylinker ~~having the sequence CGTACCCCGG CGGTCGACC~~. Nucleotides 2023 to 4154 of pUGN81-3 corresponded to nucleotides 2551 to 4682 of plasmid pBI101 (Clontech, Palo Alto, CA) followed by a polylinker ~~having the sequence ATCGGCAATT AAGCTTGCAT GCCTGCAGGC CGCGCTTAAT TAA~~ which corresponded to bases 4155 to 4197 of pUGN81-3. ~~Nucleotides 4198 to 4264 of pUGN81-3 corresponded to: GCGCGCGCTT TAACGCGCGC GCATTTAAAT GCGCGCGCGC CATCGCTTGC ACATCTGCAT CGCTG.~~ Nucleotides 4265-4776 of pUGN81-3 comprised the double-enhanced 35S promoter, with nucleotides 4265 to 4516 corresponding to nucleotides 7093 to 7344 of the Cauliflower Mosaic Virus genome (Franck et al., (1980) Cell 21:285-294). Nucleotides 4525 to 4776 of pUGN81-3 were a duplication of nucleotides 4265 to 4516 with ~~the~~ linker ~~CATCGATG~~ comprising nucleotides 4517 to 4524 between the duplicated sequence. Nucleotides 4777 to 4871 of pUGN81-3 corresponded to bases 7345 through 7439 of the Cauliflower Mosaic Virus genome (Franck et al., (1980) Cell 21:285-294). Nucleotides 4872 to 4891 comprised ~~the~~ linker ~~GCGCACTCTA CAGGATCCAG~~. Nucleotides 4892 to 5001 of pUGN81-3 corresponded to nucleotides 167 to 277 of the Maize Streak Virus genome with base 187 absent (Mullineaux et al., (1984)

a1

EMBO J. 3:3063-3068). Nucleotides 5002 to 5223 corresponded to the modified first intron of the maize alcohol dehydrogenase gene (Adh1-S) (Dennis et al., (1984) Nucleic Acids Res. 12:3983-4000). The modification resulted in removal of 343 nucleotides (bases 1313 to 1656) with bases 1222 to 1312 (intron 5' end) and nucleotides 1657 to 1775 (intron 3' end) of the maize Adh1-S gene remaining. Nucleotides 5224 to 5257 of pUGN81-3 corresponded to Maize Streak Virus (MSV) nucleotides 279 to 312. Both sections of the Maize Streak Virus, hereinafter MSV, sequence comprised the untranslated leader of the MSV coat protein V2 gene, and were interrupted in plasmid pUGN81-3 by the modified Adh1 intron. Nucleotides 5258 to 5814 of plasmid pUGN81-3 corresponded to nucleotides 29 to 585 of the phosphinotricin acetyl transferase (BAR) gene of *Streptomyces hygroscopicus* (White et al., (1989) Nucleic Acids Res. 18:1062). To facilitate cloning, nucleotides 34 and 575 of the published sequence were changed from A and G to G and A, respectively. This sequence served as the selectable marker. Nucleotides 5815 to 5819 comprised ~~the~~ linker ~~GATCT~~. Nucleotides 5820 to 6089 of pUGN81-3 corresponded to nucleotides 4414 to 4683 of plasmid pBI101 (Clontech, Palo Alto, CA) followed by ~~the~~ linker sequence ~~ATCGC~~. The remaining sequence of pUGN81-3 (nucleotides 6095 to 8730) corresponded to the reverse complement of pUC19 (Yanish-Perron et al., (1985) Gene 33:103-119).

A1 Cont.

The maize expression vector, pDAB418, containied the ubiquitin promoter regulatory element driving the β-glucuronidase gene was used some expression studies. In addition this plasmid carried a second gene which served as a plant selectable marker. Plasmid pDAB418 was a 10,149 base pairs double stranded plant transformation vector composed of the following sequences in clockwise order. ~~Nucleotides 1 to 31 had the nucleotide sequence AATTCATCGA AGGGGCCCGA AGCTTGCGCG G.~~ Nucleotides 32 to 2023 of pDAB418 were the maize ubiquitin (Ubi1) promoter and first intron,

and were PCR amplified from genomic DNA of maize genotype B73 (Christensen et al., (1992) Plant Mol. Biol. 18:675-689). Nucleotides 2024 to 2042 of pDAB418 comprised ~~the~~ linker sequence ~~GCTACCCGCG GGGTCGACC~~. Nucleotides 2043 to 3894 of pDAB418 corresponded to nucleotides 2551 to 4402 of plasmid pBI101 (Clontech, Palo Alto, CA) followed by ~~the~~ linker sequence ~~TGGGGAATTG~~ (bases 3895 to 3904). Nucleotides 3905 to 4174 OF pDAB418 corresponded to 4414 to 4683 of pBI101. Nucleotides 4175 to 4192 were composed of ~~the~~ linker sequence ~~ATCGGGAATT AAGGTTCG~~. Base 4193 through 6184 was composed of a second copy of the maize ubiquitin promoter and first intron as describe above. This sequence was followed by ~~the~~ linker sequence ~~GTCGGGGATC~~ TA (6185 to 6196). Nucleotides 6197 to 6753 of plasmid pDAB418 corresponded to nucleotides 29 to 585 of the phosphinotricin acetyl transferase (BAR) gene of Streptomyces hygroscopicus (White et al., (1989) Nucleic Acids Res. 18:1062). To facilitate cloning, nucleotides 34 and 575 of the published sequence were changed from A and G to G to A, respectively. This sequence served as the selectable marker and was regulated by the maize ubiquitin promoter. Nucleotides 6754 to 6758 were composed of ~~the~~ linker ~~TAACC~~. Nucleotides 6759 through 7472 functioned as the 3' polyadenylation sequence and includes bases 21728 through 22441 from the *Agrobacterium tumefaciens* octopine Ti plasmid pTi15955 (Barker et al. (1983) Plant Mol. Biol. 2, 335-350. Sequence 7473 through 7504 was composed of ~~the~~ polylinker ~~GCAATTGATC GATATCTAGA TCTCGAGCTC GG~~. The remaining sequence of pDAB418 (nucleotides 7505 to 10149) corresponded to the reverse complement of nucleotides from the plasmid backbone derived from pUC19 (Yanish-Perron et al., (1985) Gene 33:103-119).

A1 Cont.

In Example 9, delete the paragraph at page 40, line11 to page 41, line 3, and substitute the following new paragraph:

A2

The rice expression vector, pUbiHyg, contained the maize ubiquitin promoter and first intron from the ubiquitin

gene (Ubi1) regulatory element driving the hygromycin B phosphotransferase (resistance) gene as described by Gritz and Davies, (1983) Gene 25:179-188. Plasmid pUbiHyg was a 5991 base pairs double stranded plant transformation vector composed of the following sequences in clockwise order. ~~Nucleotides 1 to 42 had the sequence AACCTTGCAT GCCTGCAGAT CTCGGCCCGC AAGCTTCCGC GC.~~ Nucleotides 43 through 2034 of pUbiHyg were the maize ubiquitin promoter and first intron, and were PCR amplified from genomic DNA of maize genotype B73 (Christensen et al., (1992) Plant Mol. Biol. 18:675-689) ~~Nucleotides 2035 to 2052 of pUbiHyg had the sequence GGTACCCCGG GGTAGACC~~. Nucleotides 2053 through 3078 of pUbiHyg corresponded to nucleotides 211 through 1236 of the the hygromycin B phosphotransferase (resistance) gene sequence (accession number K01193), with bases 2056 and 2057 of pUbiHyg modified from AA to GT to facilitate future cloning. Bases 3079 through 3097 were composed of ~~the~~ linker ~~TAAGAGCTCG AATTTCCCC~~. Bases 3098 through 3351 corresponded to nucleotides 4430 to 4683 of plasmid pBI101 (Clontech, Palo Alto, CA). The remaining sequence of pUbiHyg (nucleotides 3352 to 5991) corresponded to nucleotides from the plasmid backbone, (Yanish-Perron et al., (1985) Gene 33:103-119).

A2

In Example 11, delete the paragraph from page 42, line 21, to page 43, line 34, and substitute the following new paragraph:

The expression vector, pSMGN179-3, contained a modified derivative of the chimeric regulatory regions from the *Agrobacterium tumefaciens* opine synthase genes described by Gelvin and Hauptmann, (1995) Patent WO 95/14098 driving the β-glucuronidase gene. Plasmid pSMGN179-3 was a 5541 base pairs double stranded plant transformation vector composed of the following sequences in clockwise order: nucleotides 1 to 16 had the multiple cloning sequence from the plasmid backbone, pUC19 (Yanish-Perron et al., (1985) Gene 33:103-119) ~~AATTCGAGCT CGCTAC~~. Nucleotides 17 to 57 of pSMGN179-3

A3

were composed of linker fragment ~~having the sequence CGGGCCCCCC CTCGACGTCG ACGGTATCGA TAAGCTTGAT C~~. Bases 58 through 277 of pSMGN179-3 corresponded to the reverse complement of bases 13774 through 13993 of *Agrobacterium tumefaciens* Ti plasmid pTi15955 T-DNA (Barker et Gal. (1983) Plant Mol. Biol. 2:335-350). These bases corresponded to upstream activating sequences from the octopine synthase (ocs) gene. Bases 278 through 304 corresponded to linker DNA ~~with the sequence CGAATTCGAA GCTTGGCGTC CACGTCA~~. Bases 305 through 350 corresponded to the reverse complement of bases 21475 through 21520 of *Agrobacterium tumefaciens* Ti plasmid pTi15955 T-DNA (Barker et Gal. (1983) Plant Mol. Biol. 2:335-350). Bases 351 through 737 of pSMGN179-3 were composed of the reverse complement of bases 20128 through 20514 from *Agrobacterium* b-glucuronidase gene *tumefaciens* Ti plasmid pTi15955 T-DNA (Barker et Gal. (1983) Plant Mol. Biol. 2:335-350). The sequence of pSMGN179-3 from 305 through 737 included the ΔMAS promoter described by Gelvin and Haupman, (1995) Patent WO 95/14098. Bases 738 through 763 of pSMGN179-3 contain linker sequence ~~CTCTAGAACT AGTGGATCGC TCGACG~~. Bases 58 through 763 of pSMGN179-3 comprised the promoter and untranslated leader regulatory fusion as given in SEQ ID NO 1. Nucleotides 764 to 2615 of pSMGN179-3 corresponded to nucleotides 2551 to 4402 of plasmid pBI101 which encoded the β-glucuronidase gene (Clontech, Palo Alto, CA)(Jefferson et al., (1986) Plant Mol. Biol. Rep. 83(22):8447-8451). Bases 767 through 769 were modified from TTA to GTC. The β-glucuronidase gene was followed by ~~the~~ polylinker sequence ~~TGGGCAATTG~~, corresponding to bases 2616 to 2625 of pSMGN179-3. Bases 2626 through 2895 corresponded to 4414 to 4683 of pBI101 which contained the sequence from the nopaline synthase 3′ untranslated regions (Clontech, Palo Alto, CA)(Jefferson et al., (1986) Plant Mol. Biol. Rep. 83(22):8447-8451). The remaining sequence of pSMGN179-3 (nucleotides 4684 to 5541) corresponded the reverse complement of nucleotides from the

A3 Cont.

A3 cont.

plasmid backbone which was derived from pUC19 (Yanish-Perron et al., (1985) Gene 33:103-119).

In Example 13, delete the paragraphs from page 44, line 37, to page 54, line 24, and substitute the following amended paragraphs:

A4

Plasmid pDAB305 was a 5800 bp plasmid that harbored a promoter containing a tandem copy of the Cauliflower Mosaic Virus 35S enhancer (35S), a deleted version of the *Adh1* intron 1, and the untranslated leader from the Maize Streak Mosaic Virus Coat Protein fused to the β-glucuronidase gene, which was then followed by the *nos* 3'UTR. It was made as follows:

The starting material was plasmid pUC13/35S(-343) as described by Odell *et al.* (1985 Nature 313:810-812). This plasmid comprised, starting at the 3' end of the Sma I site of pUC13 (Messing, 1983 *in* Methods in Enyzmology, Wu, R. Ed. 101:20-78), and reading on the strand contiguous to the noncoding strand of the *lacZ* gene of pUC13, nucleotides 6495 to 6972 of CaMV, followed by ~~the~~ linker sequence ~~CATCGATG~~ (which encodes a Cla I recognition site), followed by CaMV nucleotides 7089 to 7443, followed by ~~the~~ linker sequence ~~CAAGCTTG~~, the latter sequence comprising the recognition sequence for Hind III, which was then followed by the remainder of the pUC13 plasmid DNA.

The plasmid pUC13/35S(-343) DNA was digested with Cla I and Nco I, the 3429 base pair (bp) large fragment was separated from the 66 bp small fragment by agarose gel electrophoresis, and then purified by standard methods. The DNA was digested with Cla I, and the protruding ends were made flush by treatment with T4 DNA polymerase. The blunt-ended DNA was then ligated to synthetic oligonucleotide linkers ~~having the sequence CCCATGGG~~, which included an Nco I recognition site. The ligation reaction was transformed into competent *E. coli* cells, and a transformant was identified that contained a plasmid (named pOO#1) that had an Nco I site positioned at the former Cla I site. DNA of

pOO#1 was digested with Nco I and the compatible ends of the large fragment were religated, resulting in the deletion of 70 bp from pOO#1, to generate intermediate plasmid pOO#1 NcoΔ.

The plasmid pOO#1 NcoΔ DNA was digested with EcoR V, and the blunt ends were ligated to Cla I linkers having the sequence CATCGATG. An *E. coli* transformant harboring a plasmid having a new Cla I site at the position of the previous EcoR V site was identified, and the plasmid was named pOO#1 NcoΔ RV>Cla. This DNA was then digested with Cla I and Nco I, and the small (268 bp) fragment was purified from an agarose gel. This fragment was then ligated to the 3429 bp Cla I/Nco I fragment of pUC13/35S(-343) prepared above, and an *E. coli* transformant that harbored a plasmid having Cla I/Nco I fragments 3429 and 268 bp was identified. This plasmid was named pUC13/35S En.

The plasmid pUC13/35S En DNA was digested with Nco I, and the protruding ends were made blunt by treatment with T4 DNA polymerase. The treated DNA was then cut with Sma I, and was ligated to Bgl II linkers ~~having the sequence CACATCTC~~. An *E. coli* transformant that harbored a plasmid in which the 416 bp Sma I/NcoI fragment had been replaced with at least two copies of the Bgl II linkers was identified, and named p35S En^2. The DNA structure of p35S En^2 was as follows: Beginning with the nucleotide that follows the third C residue of the Sma I site on the strand contiguous to the noncoding strand of the *lacZ* gene of pUC13; ~~the~~ linker sequence ~~CACATCTGCA GATCTGCATC GCCCATC~~, followed by CaMV nucleotides 7090 to 7344, followed by the Cla I linker sequence ~~CATCGATC~~, followed by CaMV nucleotides 7089 to 7443, followed by the Hind III linker sequence ~~CAAGCTT~~, followed by the rest of pUC13 sequence. This structure had the feature that the enhancer sequences of the CaMV 35S promoter, which laid in the region upstream of the EcoR V site in the viral genome (bases 7090 to 7344), had been duplicated. This promoter construct incorporated the native 35S transcription start site, which was 11

nucleotides upstream of the first A residue of the Hind III site.

For plasmids utilizing the 35S promoter and the *Agrobacterium* NOS Poly A sequences, the starting material for the first construct was plasmid pBI221, purchased from CLONTECH (Palo Alto, CA). This plasmid contained a slightly modified copy of the CaMV 35S promoter. Beginning at the 3' end of the Pst I site of pUC19 (Yanisch-Perron et al., 1985 Gene 33:103-119), and reading on the same strand as that which encodes the *lacZ* gene of pUC19, the sequence was comprised of ~~the~~ linker nucleotides ~~GTCGAC~~, followed by CaMV nucleotides 6605 to 7439 followed by ~~the~~ linker sequence ~~GGGGACTCTA GAGGATCCCC GGGTGGTCAG TGCGTT~~. These bases were then followed by 1809 bp comprising the coding sequence of the *E. coli uidA* gene, which encodes the ß-glucuronidase (GUS) protein, and 55 bp of 3' flanking bases that are derived from the *E. coli* genome (Jefferson, 1986 Proc. Natl. Acad. Sci. 83:8447-8451), followed by the Sac I linker sequence ~~GAGCTC~~, which was then followed by ~~the~~ linker sequence ~~GAATTTGCGC~~. These bases were followed by the RNA transcription termination/polyadenylation signal sequences derived from the *Agrobacterium tumefaciens* nopaline synthase (NOS) gene, and comprised the 256 bp Sau3A I fragment corresponding to nucleotides 1298 to 1554 of DePicker *et al.* (1982 J. Molec. Appl. Genet. 1:561-573), followed by two C residues, the Eco RI recognition sequence ~~GAATTC~~, and the rest of pUC19.

The plasmid pBI221 DNA was digested with EcoR I and BamH I, and the 3507 bp fragment was purified from an agarose gel. Plasmid pRAJ275 (CLONTECH) DNA was digested with EcoR I and Sal I, and the 1862 bp fragment was purified from an agarose gel. These two fragments were mixed together, and complementary synthetic oligonucleotides ~~having the sequence GATCCGCATC GC and TCGACGCATC GC~~ were added. The fragments were ligated together, and an *E.coli* transformant harboring a plasmid having the appropriate DNA structure was identified by restriction enzyme analysis.

DNA of this plasmid, named pKA881, was digested with Bal I and Eco RI, and the 4148 bp fragment was isolated from an agarose gel. DNA of pBI221 was similarly digested, and the 1517 bp Eco RI/Bal I fragment was gel purified and ligated to the above pKA881 fragment, to generate plasmid pKA882. Then pKA882 DNA was digested with Sac I, the protruding ends were made blunt by treatment with T4 DNA polymerase, and the fragment was ligated to synthetic BamH I linkers ~~having the sequence CGGATCCG~~. An *E.coli* transformant that harbored a plasmid having BamH I fragments of 3784 and 1885 bp was identified and named pKA882B. Following, pKA882B DNA was digested with BamH I, and the mixture of fragments was ligated. An *E.coli* transformant that harbored a plasmid that generated a single 3783 bp fragment upon digestion with BamH I was identified and named p35S/NOS. This plasmid had the essential DNA structure of pBI221, except that the coding sequences of the GUS gene had been deleted. Therefore, CaMV nucleotides 6605 to 7439 were followed by ~~the~~ linker sequence ~~GGGGACTCTA GAGGATCCCG AATTTCCGG~~. The linker sequence was then followed by the NOS Polyadenylation sequences and the rest of pBI221.

A4 cont.

The plasmid p35S/NOS DNA was digested with EcoR V and Pst I, and the 3037 bp fragment was purified and ligated to the 534 bp fragment obtained from digestion of p35S En^2 DNA with EcoR V and Pst I. An *E. coli* transformant was identified that harbored a plasmid that generated fragments of 3031 and 534 bp upon digestion with EcoR V and Pst I, and the plasmid was named p35S En^2/NOS. This plasmid contained the duplicated 35S promoter enhancer region described for p35S En^2, the promoter sequences being separated from the NOS polyadenylation sequences by linker sequences that included unique Xba I and BamH I sites.

The MSV genomic sequence was published by Mullineaux *et al.*, (1984 EMBO J. 3:3063-3068), and Howell (1984 Nucleic Acids Res. 12:7459-7375), and the transcript was described by Fenoll *et al.* (1988 EMBO J. 7:1589-1596). The entire sequence, comprised 154 bp and was constructed in three

stages by assembling blocks of synthetic oligonucleotides. First, complementary oligonucleotides ~~having the sequence CATCCACCTC AACCCTCCAC AACCCACATC CACCCACCAC CTCATATTTC CTCCACA and ACCTTCTCCA CCAAATATCA CCTCCTCCCT CCATCTCCCT TCTCCACCCT TCACCTC~~ were synthesized and purified by standard procedures. Annealing of these nucleotides into double-stranded structures left 4-base single stranded protruding ends compatible with those generated by BamH I on one end of the molecule ~~(GATC)~~, and with Hind III-generated single stranded ends on the other end of the molecule ~~(ACCT)~~. Such annealed molecules were ligated into plasmid pBluescript SK(-) [hereinafter called pBSK; Stratagene Cloning Systems, La Jolla, CA], that had been digested with BamH I and Hind III. The sequence of these oligonucleotides was such that when ligated onto the respective BamH I and Hind III sticky ends, the sequences of the respective recognition sites were maintained. An *E. coli* transformant harboring a plasmid containing the oligonucleotide sequence was identified by restriction enzyme analysis, and the plasmid was named pMSV A.

A4 cont.

Complementary oligonucleotides ~~having the sequences ACCTCTCCAT AGCACCAACC CTATCCCTAA TATACCAGCA CCACCAAGTC AGCCCAATCC CCCC and TCCACCCCCC ATTCCCCTCA CTTCCTCCTC CTCCTATATT ACCCATACCC TTCCTCCTAT CCAC~~ were synthesized and purified by standard procedures. Annealing of these nucleotides into double-stranded structures left 4-base sticky ends that were compatible with those generated by Hind III on one end of the molecule ~~(ACCT),~~ and with Sal I-generated sticky ends on the other end of the molecule (TCGA). The sequence of these oligonucleotides was such that when ligated onto the Hind III sticky ends the recognition sequence for Hind III was destroyed.

DNA of pMSV A was digested with Hind III and Sal I, and was ligated to the above annealed oligonucleotides. An *E. coli* transformant harboring a plasmid containing the new oligonucleotides was identified by restricition enzyme site mapping, and was named pMSV AB.

Complementary oligonucleotides ~~having the sequences CCCGGCCATT TGTTCCAGGC ACGCGATAAG CATTCAGCCA TGCGATATCA AGCTTGGATC CC and TCGAGGGATC CAAGCTTCAT ATCCCATGCG TCAATGCTTA TCCCGTGCGT CGAACAAATC GC~~ were synthesized and purified by standard procedures. These oligonucleotides incorporate bases that comprise recognition sites for Nco I, EcoR V, Hind III, and BamH I. Annealing of these nucleotides into double-stranded structures left 4-base sticky ends that were compatible with those generated by Xma I on one end of the molecule ~~(CCGG)~~ , and with Xho I-generated sticky ends on the other end of the molecule ~~(TCGA)~~ . Such annealed molecules were ligated into pMSV AB DNA that had been digested with Xma I and Xho I. An *E. coli* transformant harboring a plasmid containing the oligonucleotide sequence was identified by restriction enzyme analysis, and DNA structure was verified by sequence analysis. The plasmid was named pMSV CPL. Together, these comprised the 5' untranslated leader sequence ("L") of the MSV coat protein ("CP") gene. These corresponded to nucleotides 167 to 186, and 188 to 317 of the MSV sequence of Mullineaux *et al.*, (1984), and were flanked on the 5' end by the BamH I linker sequence ~~GGATCCAG~~, and on the 3' end by ~~the~~ linker sequence ~~GATATCAAGC TTGCATCCG~~. An A residue corresponding to base 187 of the wild type MSV sequence was inadvertently deleted during cloning.

A4 cont.

The plasmid pMSV CPL DNA was digested at the Sma I site corresponding to base 277 of the MSV genomic sequence, and the DNA was ligated to Bgl II linkers ~~having the sequence CAGATCTG~~. An *E. coli* transformant harboring a plasmid having a unique Bgl II site at the position of the former Sma I site was identified and verified by DNA sequence analysis, and the plasmid was named pCPL-Bgl.

The starting material in construction of a deleted version of the maize alcohol dehydrogenase 1 (Adh1) intron 1 is plasmid pVW119, which was obtained from V. Walbot, Stanford University, Stanford, CA. This plasmid contained the DNA sequence of the maize Adh1.S gene, including intron

1, from nucleotides 119 to 672 [numbering of Dennis *et al.* (1984 Nucleic Acids Res. 12:3983-4000)], and was described in Callis *et al.* (1987 Genes and Develop. 1:1183-1200). ~~In pVW119, the sequence following base 672 of Dennis et al. (1984) was GACGGATCC.~~ The entire intron 1 sequence, with 14 bases of exon 1, and 9 bases of exon 2, was obtained from this plasmid on a 556 bp fragment following digestion with Bcl I and BamH I.

The plasmid pSG3525a(Pst) DNA was digested with BamH I and Bcl I, and the 3430 bp fragment was purified from an agarose gel. DNA of plasmid pVW119 was digested with BamH I and Bcl I, and the gel purified fragment of 546 bp was ligated to the 3430 bp fragment. An *E. coli* transformant was identified that harbored a plasmid that generated fragments of 3430 and 546 upon digestion with BamH I and Bcl I. This plasmid was named pSG AdhA1.

The DNA of pSG AdhA1 was digested with Hind III and with Stu I. The ends were made flush by T4 DNA polymerase treatment and then ligated. An *E. coli* transformant that harbored a plasmid lacking Hind III and Stu I sites was identified and the DNA structure was verified by sequence analysis. The plasmid was named pSG AdhA1Δ. In this construct, 344 bp of DNA had been deleted from the interior of the intron 1. The loss of these bases did not affect splicing of this intron. The functional intron sequences ware obtained on a 213 bp fragment following digestion with Bcl I and BamH I.

DNA of plasmid pCPL-Bgl was digested with Bgl II, and the linearized DNA was ligated to the 213 bp Bcl I/BamH I fragment containing the deleted version of the Adh1.S intron 1 sequences from pSG AdhA1Δ. An *E. coli* transformant was identified by restriction enzyme site mapping that harbored a plasmid that contained the intron sequences ligated into the Bgl II site, in the orientation such that the Bgl II/Bcl I juncture was nearest the 5' end of the MSV CPL leader sequence, and the Bgl II/ BamH I juncture was nearest the 3' end of the CPL. This orientation was confirmed by DNA

sequence analysis. The plasmid was named pCPL A1I1Δ. The MSV leader/intron sequences was obtained from this plasmid by digestion with BamH I and Nco I, and purification of the 373 bp fragment.

Construction of plant expression vectors based on the enhanced 35S promoter, the MSV CPL, and the deleted version of the Adh1 intron 1 was as follows. DNA of plasmid p35S En^2/NOS was digested with BamH I, and the 3562 bp linear fragment was ligated to a 171 bp fragment prepared from pMSV CPL DNA digested with BamH I. This fragment contained the entire MSV CPL sequence. An *E. coli* transformant was identified by restriction enzyme site mapping that harbored a plasmid that contained these sequences in an orientation such that the Nco I site was positioned near the NOS Poly A sequences. This plasmid was named p35S En^2 CPL/NOS. It contained the enhanced version of the 35S promoter directly contiguous to the MSV leader sequences such that the derived transcript included the MSV sequences in its 5' untranslated portion.

A4 cont.

The DNA of plasmid pKA882 was digested with Hind III and Nco I, and the large 4778 bp fragment was ligated to an 802 bp Hind III/Nco I fragment containing the enhanced 35S promoter sequences and MSV leader sequences from p35S En^2 CPL/NOS. An *E. coli* transformant harboring a plasmid that contained fragments of 4778 and 802 bp following digestion with Hind III and Nco I was identified, and named pDAB310. In this plasmid, the enhanced version of the 35S promoter was used to control expression of the GUS gene. The 5' untranslated leader portion of the transcript contains the leader sequence of the MSV coat protein gene.

DNA of plasmid pDAB310 was digested with Nco I and Sac I. The large 3717 bp fragment was purified from an agarose gel and ligated to complementary synthetic oligonucleotides ~~having the sequences CGGTACCTCGACTTAAC and CATGGTTAACTCGACGTACCGACGT~~. These oligonucleotides, when annealed into double stranded structures, generated molecules having sticky ends compatible with those left by

Sac I, on one end of the molecule, and with Nco I on the other end of the molecule. In addition to restoring the sequences of the recognition sites for these two enzymes, new sites were formed for the enzymes Kpn I ~~(GGTACC)~~, Xho I ~~(CTCGAG)~~, and Hpa I ~~(GTTAAC)~~. An *E. coli* transformant was identified that harbored a plasmid that contained sites for these enzymes, and the DNA structure was verified by sequence analysis. This plasmid was named pDAB1148.

DNA of plasmid pDAB1148 was digested with Bam HI and Nco I, the large 3577 bp fragment was purified from an agarose gel and ligated to a 373 bp fragment purified from pCPL A1I1Δ following digestion with Bam HI and Nco I. An *E. coli* transformant was identified that harbored a plasmid that generated fragments of 3577 and 373 bp following digestion with BamH I and Nco I, and the plasmid was named pDAB303. This plasmid has the following DNA structure: beginning with the base after the final G residue of the Pst I site of pUC19 (base 435), and reading on the strand contiguous to the coding strand of the *lacZ* gene, ~~the~~ linker sequence ~~ATGTGCATGG GTG~~, nucleotides 7093 to 7344 of CaMV DNA, ~~the~~ linker sequence ~~CATGGATG~~, nucleotides 7093 to 7439 of CaMV, ~~the~~ linker sequence ~~GGGGACTCTA GAGGATCCAG~~, nucleotides 167 to 186 of MSV, nucleotides 188 to 277 of MSV, a C residue followed by nucleotides 119 to 209 of Adh1.S, nucleotides 555 to 672 of maize Adh1.S, ~~the~~ linker sequence ~~GAGGCATGTG~~, nucleotides 278 to 317 of MSV, ~~the~~ polylinker sequence ~~GTTAACTCGA GGTACCGAGC TCGAATTTCC GC~~ containing recognition sites for Hpa I, Xho I, Kpn I, and Sac I, nucleotides 1298 to 1554 of NOS, and a G residue followed by the rest of the pUC19 sequence (including the EcoR I site).

A4 cont

DNA of plasmid pDAB303 was digested with Nco I and Sac I, and the 3939 bp fragment was ligated to the 1866 bp fragment containing the GUS coding region prepared from similarly digested DNA of pKA882. The appropriate plasmid was identified by restriction enzyme site mapping, and was named pDAB305. This plasmid had the enhanced promoter, MSV

leader and Adh1 intron arrangement of pDAB303, positioned to control expression of the GUS gene.

The plasmid pDAB305 contained an enhanced 35S promoter with additional 3' sequences and embodied as nucleotides 7093 to 7344 of CaMV DNA, ~~the~~ linker sequence ~~CATCGATG~~, nucleotides 7093 to 7439 of CaMV, ~~the~~ linker sequence ~~CGGCACTCTA GACGATCCAC~~, nucleotides 167 to 186 of MSV, nucleotides 188 to 277 of MSV, a C residue followed by nucleotides 120 to 210 of maize Adh1.S, nucleotides 555 to 672 of maize Adh1.S, ~~the~~ linker sequence ~~GACCGATCTG~~, nucleotides 278 to 317 of MSV, and a G residue that represents the final base of an Nco I recognition sequence, ~~CCATGG~~. As above, the GUS translational start codon was part of the Nco I site. Transcripts from this promoter contain as the 5' untranslated leader essentially the MSV coat protein leader sequence, into which has been inserted a deleted version of the maize Adh1.S intron 1.

Q4 cont.